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ABSTRACTS FROM THE ORIGINAL PAPERS.

On the Distribution of Azotobacter in Relation to the Reaction of Soils in Japan.

By Unokichi. Yamagata.

This investigation was made to deal with the distribution of various kinds of Azotobacter in three hundred samples* of soils collected in Japan, and with the relation of their growth to the reaction of soils, especially with the concentration of hydrogen-ion.

Three trypes of Azotobacter; viz., Azotobacter Chroöcoccum, Azotobacter Beijerinckii, and Azotobacter Vinelandii were isolated from one hundred samples and they were cultured in pure state according to the method of the author and Aoi. The distribution of Azotobacter in soils of cultivated land in Japan is shown in the following table:

Field.	Number of Samples examined.	Number of Samples containing Azotobacter.	Number of Samples containig Azotobacter		
			Chroöcoccum.	Beijerinckii.	Vinelandii.
Paddy.	137	45	25	15	5
Upland.	163	55	41	4	10

Districts where samples collected.	Number of Samples examined.	Number of Samples containing Azotobacter.	Percentage of Samples containing Azotobacter.
Hokkaido.	10	1	10
Tohoku.	80	8	10
Hokuriku	10	1	10
Tosan.	20	3	15
Kwanto.	42	17	40
Tokai.	19	7	37
Kinki.	10	4	40
Chugoku.	25	12	48
Shikoku.	18	6	33

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*These Samples represent various cultivated soils from the extreme south to the north of Japan.

Kiushu.	38	24	63
Okinawa.	6	4	66
Taiwan.	22	13	59
Sum	300	100	33

From these results, it is clearly seen that azotobacter is distributed in soils of paddy as well as in upland fields, and also there is a close relation between the distribution and the climatic condition of the districts. The number of percent of soil-samples containing azotobacter to the number of soil-samples examined was only 10 in the northern part of Japan, but more than 60 in the southern part. Azotobacter Chroöcoccum and Azotobacter Vinelandii were found mostly in the soil of upland field, while Azotobacter Beijerinckii in the soil of paddy field.

The distribution of these organisms and the reaction of the soils examined, are as follows:

Reaction of Soil.	Number of Soil-samples examined.	Number of Azotobacter contained in Soils		
		Chroöcoccum.	Beijerinckii.	Vinelandii.
Acid	119	0	0	0
neutral	76	3	11	0
slightly alkaline	62	26	20	0
alkaline	43	37	0	3
Sum	300	66	31	3

Thus, among these three, Azotobacter chroöcoccum was most largely distributed, and especially in the alkaline soils; Azotobacter Beijerinckii was less common and found in nearly neutral soils; and Azotobacter Vinelandii was least common and presented in only the alkaline soils. Azotobacter was always found in the soils containing wood-ash, calcium carbonate, or sea shells.

The properties of the organisms isolated are shown in the table below.

	Chroöcoccum type	Beijerinckii type	Vinelandii type
Form and size.	short plump rod, gen. in pairs $2.3\mu \times 3.4\mu$	large oval $3.3\mu \times 4.7\mu$	oval $1.8\mu \times 3.4\mu$
Motility	motile, in young culture only	no	motile
Coloration on Ashby agar 28 to 30°	A. white B. brownish black	white sulphur-yellow	white greenish yellow; soluble
Coloration in Ashby solution 28 to 30°	A. white, turbid B. brownish black surface film.	white, turbid white, pellicle formation.	greenish yellow yellowish pink

Colony on Ashby agar plate 28 to 30°	round, pasty, dark concentric ring in the center	somewhat round moist, wrinkled surface	round, semitransparent

The quantities of nitrogen fixed by these organisms per one gram of mannit were as follows:

Chroöcoccum type	Bejerinckii type	Vinelandii type
6.28 mg.	4.87 mg.	10.01 mg.

These organisms were cultured in Ashby solution of different P_H, and the optimum P_H and limiting P_H were noted. For the determination of P_H, the electrometric method that was described by Itano and modified later slightly by the same author was used, and the P_H of the solution was determined by titrating electrometrically with $N/10$ HCl and $N/10$ $NaOH$. One cubic centimeter of forty-eight hour old culture in Ashby solution of P_H 7.0 was trasferred into 19 c.c. of the culture solution of respective P_H, so that total volum became 20 c.c. in every case, and the number of bacteria in 20 c.c. medium was counted after 96 hours. Also the rate of growth was noted after 48, 96 and 240 hours, and the final P_H of the medium was determined. The result obtained was as follows:

Azotobacter Chroöcoccum:. Better growth took place in media P_H 7.0, and the optimum P_H seemed to lie between 7.45 and 7.60, a marked beneficial effect of $CaCO_3$ on the growth was indicated by the fact that in the control Ashby solution with $CaCO_3$, the organism grew to 484 millions, while the maximum growth was 176 millions in the others.

Azotobacter Beijerinckii: The rate of growth was more markedly influenced according to the different P_H values than in the case of other azotobacter, for instance, at P_H 7.0 a good growth of Azotobacter Beijerinckii took place at the end of forty-eight hours, while at all other reactions the organisms were inhibited, this organism was not influenced by the presence of $CaCO_3$ or at least not so much as others, the optimum P_H seemed to lie between P_H 6.65 to 6.75.

Azotobacter Vinelandii: The behavior of this organism was similar to that of Azotobacter Chroöcoccum except the inhibitory action by P_H below 7 was slightly greater, much greater beneficial effect of $CaCO_3$ was noted; viz., 944 millions in the control and 72 millions in the medium of original P_H 8.6 and of final P_H 7.0.

Further, the acidity of soils was defermined by the method of Christensen comparing with the method of Itano's electrometric method, and the fact that the reaction of soils containing azotobacter was alkaline in most cases and that no azotobacter was found in the soil of acid reaction was observed. The following table shows the relation between the reaction of soils and the distribution of Azotobacter:

Reaction	P_H	Number of Soils examined	Number of Soils containing Azotobacter	Azotobacter		
				Chroōcoccum	Beijerinckii	Vinelandii
acid	less than 5.9	29	0	0	0	0
weak acid slightly acid	6.0–6.4	90	0	0	0	0
neutral slightly alkaline	6.5–7.2	76	8	3	5	0
weak alkaline	7.3–7.7	62	52	26	14	12
alkaline	more than 7.8	43	40	37	0	3

Summary.

1. The distribution of Azotobacter in various cultivated soils in Japan was examined and it was found that this organism was present more in the soils of warmer climate region. The precentage of this soils containing azotobacter all was about 30.
2. Azotobacter was found in the soils containing lime, wood-ash or sea-shells, and also in most soils of alkaline reaction ($P_H=7.3-7.8$) but no azotobacter in the soils of acid reaction ($P_H=$less than 6.4).
3. Three types of Azotobacter; viz., A. Chroöcoccum, A. Beijerinckii and A. Vinelandii were isolated from the sample soils.
4. The quantities of nitrogen fixed by these organisms are as follows:

	N fixed per gram of mammit.
A. Chroöcoccum.	6.28 mg.
A. Beijerinckii.	4.87 mg.
A. Vinelandii.	11.01 mg.

5. Two types of Azotobacter chroöcoccum and Azotobacter vinelandii were found mostly in the soils of weak alkaline or of alkaline reaction ($P_H=$ 7.3–7.8), while the Azotobacter Beijerinckii. in the soils of neutral, or in weak alkaline reaction ($P_H=6.5-7.7$).

6 Azotobacter was isolated from the soils of paddy as well as from that of upland field. Two types of A. chroöcoccum and A. vinelandii were found mostly in upland soils, while A. Beijerinckii in paddy soils.

7. A pure culture of Azotobacter has easily made according to the method of the author and Aoi.
8. The author has used the apparatus schemed by Itano for determining hydrogen-ion concentration.
9. For the purpose to compare the rate of growth of these organisms in culture media of different P_H, Ashby solutions of different P_H were prepared by electometrical titration.
10. The behavior of Azotobacter chroöcoccum was similar to that of Azotobacter vinelandii, and they are not influenced by alkaline reaction, but the latter was more influenced by acid reaction than the former. Azotobacter Beijerinckii was more greatly influenced at different P_H values than in the case of other two organisms.
11. The optimum and limiting values of P_H for the growth of these organisms were as follows:

Types	Optimum value	Limiting value
A. Chroöcoccum.	7.6–8.3	5.8
A. Beijerinckii.	6.8–7.4	5.8
A. Vinelandii.	7.6–8.3	5.9

12. The beneficial effect of calcium carbonate was noted for the growth of Azotobacter Chroöcoccum and Azotobacter Vinelandii, especially for the latter. Azotobacter Beijerinckii was not so much influenced by this alkaline substance.

Finally, the author desires to express his sincere thanks to Prof. K. Aso and Dr. Itano for their kind suggestions, and also to Dr. S. Saito and C. Matsuoka for their valuable assistance during this investigation.

On the Occurrence of a New Sulphur Compound in Yeast.

by UMETARŌ SUZUKI, SATORU ŌDAKE and TAKAJIRO MORI.

Ten years ago, one of the authors (U. Suzuki) isolated a basic compound from the alcoholic extract of yeast and gave the empirical formula $C_9H_{14}N_4O_4$ to it. It was a colourless prism, melting at 208° (not corr). The aqueous solution was nearly neutral to litmus, and possessed a slightly bitter taste. It was precipitated with phosphotungstic or phosphomolybdic acid, but not with basic lead acetate. It formed also the crystalline picrate, melting at 183°. (not corr.) (Jour. Tokio Chem. Soc., Vol. 34, p.p. 1134–1135. June. 1914)

Further studies on this substance were interrupted for a long time; however, quite recently it was found by the authors that it contains sulphur, consequently the proposed formula must be abandoned. For such a reason the authors prepared this compound again in a large quantity and carefully investigated its chemical nature. The analysis of the free base as well as that of its picrate have shown that the empirical formula should be $C_{11}H_{15}N_5SO_3$.

The isolation of this compound was carried out as follows:—

The alcoholic extract of yeast was evaporated in vacuum. The resulting syrup was dissolved in a little water, and a concentrated tannin solution was added to it. The precipitate thus formed was rubbed with baryta water and filtered. The filtrate was freed from an excess of baryta, and evaporated to a syrup again, and treated with 50 % aceton, whereby a portion remained undissolved. The dissolved portion was evaporated to a thick brown syrup which possessed strong antineuritic properties. After long standing, the sulphur compound separated out from this syrup in colourless crystals, which was recrystallsed from the water solution.

When this base is boiled with dilute acid it is easily hydrolysed, thereby adenine ($C_5H_5N_5$) and a thio-sugar being formed. Although the authors have not yet succeeded to isolate the free thio-sugar in crystalline state, but it was isolated as a nice crystalline phenylosazon, having the melting point of 158–159°, and the analysis of the latter compound agreed with the formula $C_{18}H_{22}N_4SO_2$, that is, phenylosazon of a thio-sugar having the formula $C_6H_{12}SO_4$.

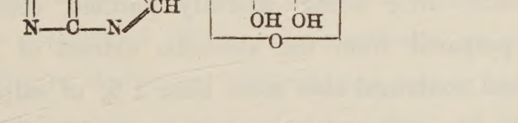
The free base gives the pentose reaction with Bial's reagent, and it gives also furfurol reaction when it is boiled with hydrochloric or sulphuric acid. By evaporating the base with strong nitric acid, the crystals of hypoxanthin nitrate were obtained, which was formed from adenine; the oxidation proceeded more energetically when a trace of vanadium oxide was added to the nitric acid solution, and besides hypoxanthin, crystals of xanthin nitrate were obtained. By shaking the mother liquid of hypoxanthin or xanthin with ether, and after evaporating the ethereal solution, colourless prisms of oxalic acid were obtained. Furthermore, in the remaining aqueous solution the presence of some sulphuric acid could be detected, this shows that a part of sulphur present in the base was oxidized to sulphuric acid.

When it is boiled with dilute hydrochloric acid, and barium chloride is added to it, no precipitate is formed, this indicates that the sulphur present does not exist in the sulphate form. In a cold dilute alkaline solution it gives no reaction with sodium nitroprusside, but it gives a dark red colour when it is previously boiled with concentrated alkali before adding sodium nitroprusside.

When this base is boiled with dilute hydrochloric acid, adding a piece of metallic zinc, there develops a gas having a strong pungent odour, which gives a white turbidity when it is introduced into an aqueous solution of mercuric nitrate or mercuric chloride. As neither the presence of hydrogen sulphide nor mustard oil could be detected in this case, it is most probable that this gas chiefly consists of mercaptane or dimethyl sulphide.

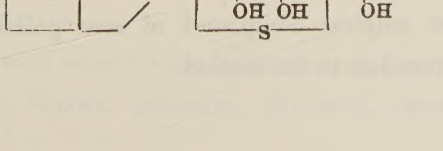
From these observations, the authors concluded that the basic substance is a compound of adenine and a thio-sugar (probably thio-methyl pentose) having one of the following formulae :—

(I)



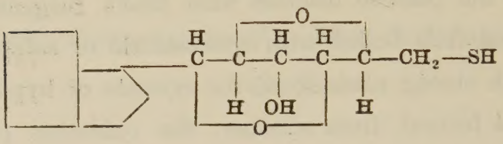
$=C_{11}H_{15}N_5SO_3$

(II)



$=C_{11}H_{15}N_5SO_3$

(III)



$=C_{11}H_{13}N_5SO_3$

Of the formulae above given, the formula (I) seems to agree best with various facts. Further studies will be required to decide whether this assumption is correct. Not only the occurrence of thio-sugar in organisms is quite new, but its combination with adenine is of special interest from the biochemical point of view, because it has a great resemblance with adenosine, a compound of adenine with ribose, which forms an essential part of nucleic acid.

Among the sulphur compounds in animal organisms cystin, cystein, taurin, and taurocholic acids are most popular ones. Further, chondroitic acid forms an essential part of cartilages. The etherial sulphates of urine are to be considered as the end product of sulphur metabolism. Mustard oil groups are widely distributed in plant. Recently, a sulphur derivative of histidine betaine, i. e. ergothionin was isolated by Tanret from ergot. J. H. Müller has reported a new amino acid containing sulphur, which is probably ethyl ester of cystein. $C_2H_5S\text{-}CH_2CH\text{-}NH_2\text{-}COOH$. (J. H. Müller: J. Biol, Chem. **56**. No. 1, pp. 157–169). The brain substance of animals contains also not inconsiderable sulphur, but nothing is known about its chemical nature, though it is generally believed that it plays just so important rôle like phosphoric acid in the nervous tissues. The authors suppose that the sulphur compound discovered by them has also a certain function in the yeast cells.

It may also be mentioned here that the alcoholic extract of rice polishings after long standing, always produces an amorphous brown precipitate. B. Suzuki has recently investigated this precipitate, and found that it has a strong stimulating action upon the growth of yeast, though it has no antineuritic action on pigeons (J. Chem. Soc. Japan, 1924). Now it was found by the authors that this precipitate, after being carefully purified, contains a little S. The crude oryzanin prepared from the alcoholic extract of yeast by the phosphotungstic acid method contained also more than 1 % of sulphur. Though it has no direct relation with the antineuritic properties, yet it is very interesting that it is always present in oryzanin preparation of yeast and rice polishings. The question that whether the sulphur compound of rice polishings is same with that of yeast or not still remains to be settled.